Spores of lichen-forming fungi in the mycoaerosol and their relationships with climate factors

Spores of lichen-forming fungi in the mycoaerosol and their relationships with climate factors

S.E. Favero-Longo, S. Sandrone, E. Matteucci, L. Appolonia, R. Piervittori

Abstract

Fungal particulates are a dominant component of the bioaerosol, but aerobiological studies traditionally focused on a limited set of fungi having relevance as allergens or plant pathogens. This study first analyzes the occurrence of lichen meiospores in the mycoaerosol, quantitatively evaluating in the atmosphere of an alpine environment the occurrence of polar diblastic spores, unequivocally attributable to the lichen family Teloschistaceae. The analysis of air-samples collected one week per month for one year with a Hirst-type sampler displayed a low percentage occurrence of polar-diblastic spores (< 0.1%) with respect to the whole mycoaerosol, dominated by *Cladosporium*. Spearman's correlation tests on aerobiological and climatic data highlighted a strong relationship between the detection of Teloschistaceae spores and rainfall events, excluding seasonal patterns or daily rhythms of dispersion. The fact that all the air-sampled spores were attributable to the species of Teloschistaceae occurring in the site, together with laboratory observations of predominant short range dispersal patterns for polar diblastic and other lichen spores, indicated that sexual reproduction is mostly involved in the local expansion of colonization, dispersal from a long distance appearing a less probable phenomenon. These findings indicated that responses of lichen communities to climate factors, usually related to physiological processes, also depend on their influence on meiospore dispersal dynamics. Spatial limitations in dispersal, however, have to be taken into account in evaluating lichen distributional shifts as indicators of environmental changes.

1. Introduction

The mycoaerosol is one of the dominant components of air particulate matter: mass fluxes of fungal spores over land range in the different biomes between 10^{-13} and 10^{-10} kg m^{-2} s^{-1} (Sesartic and Dallafior, 2011), climate factors mainly determining production, release, transport and deposition patterns (Trejo et al., 2012). Usual concentrations of 10^3–10^4 spores m^{-3} match a high diversity of airborne fungi: molecular genetic techniques revealed more than one thousand species in approx. 10^5 m^3 of air filtered over one year in a single site of central Europe (Fröhlich-Nowoisky et al., 2009). However, aerobiological studies have been traditionally focused on a limited set of fungi which are well-known allergens or plant pathogens, and this species spectrum has not been widened following recent interest on the relevance of mycoaerosol for atmospheric processes of cloud condensation and ice nucleation (Després et al., 2012). The occurrence of large groups of fungi in the atmosphere is thus still overlooked, although their fundamental ecological role and their relevance for human interests are generally accepted, as in the case of lichens.

Lichen-forming fungi are worldwide ubiquitous in terrestrial environments and represent more than 40% of the 64,000 described species of Ascomycota (Schoch et al., 2009). Interests in lichens and their colonization dynamics range through several research fields, including the biomonitoring of air quality (Nimis et al., 2002), the control of biodeteriogenic processes on the stone cultural heritage (St. Clair and Seaward, 2004) and the analysis of vegetation successional patterns or diversity shifts as indicators of climate change (Aragón et al., 2012, Ellis and Coppins, 2010 and Raab et al., 2012). Micro- and macroclimatic conditions, in particular ambient moisture and temperature, are indeed known to control the composition of lichen communities by affecting their key physiological processes (Maphangwa et al., 2012 and Marini et al., 2011). Nevertheless, the occurrence of lichen-forming fungi in the bioaerosol has been scanty assessed,

quantified and related with climate factors (Favali et al., 2003). The flux in the air of asexual symbiotic propagules (e.g. soredia) has been relatively more documented than that of meiospores (ascospores or basidiospores) and mitospores (conidia) produced by mycobionts (see Büdel and Scheidegger, 2008 for details on the reproductive structures of lichens). The aerial concentrations of lichen soredia have been particularly quantified and characterized in terms of seasonality in the Maritime Antarctic (Marshall, 1996) and NW Spain (Tormo et al., 2001), and their dispersal patterns have been variously examined in artificial environments (Armstrong, 1994 with refs. therein). Since the 60s, the dispersal of spores by lichen ascocarps has been widely examined in controlled conditions in terms of rate, mode and distance of discharge, also evaluating seasonal variations and environmental conditioning factors (Bailey, 1976, Clayden, 1997 and Pyatt, 1973). On the other hand, in most cases, lichen spores sampled from the atmosphere cannot be distinguished from spores of non-lichenized fungi on the basis of morphology and are not sufficiently competitive in growth with respect to other fungi to be identified using cultivation-dependent techniques. It is thus a consequence that only two reports provide evidence of lichen meiospores in the bioaerosol: some spores sampled in the air over Cape Hallett (Antarctica) were attributed to *Buellia* and some spores trapped in the air of Sutton (Surrey, UK) were ascribed to *Caloplaca holocarpa* and *Caloplaca aurantia* (Bailey, 1976), but no quantification of lichen meiospores in the atmosphere is available. The polar-diblastic (syn. polarilocular) spores, however, are unequivocally attributable to the Teloschistaceae, which exclusively include lichen-forming fungi (e.g. genera *Caloplaca*, *Xanthoria*, *Teloschistes*, *Huea*) (Bailey, 1976), thus offering the possibility to quantitatively, although partially, monitor the occurrence of lichen meiospores in the mycoaerosol. This study quantitatively monitored the contribution of polar-diblastic spores to the total spore content in the atmosphere of an alpine site of NW-Italy, where different Teloschistaceae species colonize the walls of a medieval castle and the underlying bedrock. Spore amounts were evaluated with reference to meteorological parameters. We aimed to test the hypothesis that climate factors as ambient moisture and temperature, which are known to drive community compositions by influencing lichen physiology, also regulate spore dispersal processes. Moreover, we tested in the laboratory if different dispersal patterns characterize species with different spore size by simulating within a microcosm the air flow on lichenized rock surfaces.

2. Material and methods

2.1. Sampling site

The aerobiological monitoring was carried on at the court of the ruined medieval Castle of Graines, built since the XII century at 1367 m a.s.l. on the top of a steep relief located in the Ayas Valley (UTM ED50, N 5065923, E 403150; Brusson, Aosta Valley, Italy). This intra-alpine area displays a fairly dry climate regime, with annual rainfall around 800 mm and mean air temperatures ranging from 0 °C in winter to 17 °C in summer (Verger, 1992). The castle walls, about 10 m high, built with blocks of chloritoschists from the bedrock and, subordinately, of other lithotypes, extend along a perimeter of 250 m and surround an area of approx. 2000 m^2, including about 200 m^2 of natural rock outcrops and discontinuous stands of sub-alpine grassland. A lichen flora of 53 saxicolous species, mostly silicicolous and xerophytic ones, was described for the castle site (Piervittori et al., 1991). Five species of Teloschistaceae occur, namely *Caloplaca crenularia* (With.) J.R. Laundon, *Caloplaca crenulatella* (Nyl.) H. Olivier, *Caloplaca rubroaurantiaca* de Lesd, *Caloplaca saxicola* s.l. and *Xanthoria elegans* (Link.) Th. Fr., their contribution to a total lichen covers 80% on natural outcrops and 50% on the walls being about 1% (see Supplementary materials 2). Beyond their thallus morphology, these species also display significant differences in their spore-size features, with the exception of *X. elegans* and *C. saxicola* (Fig. 1).

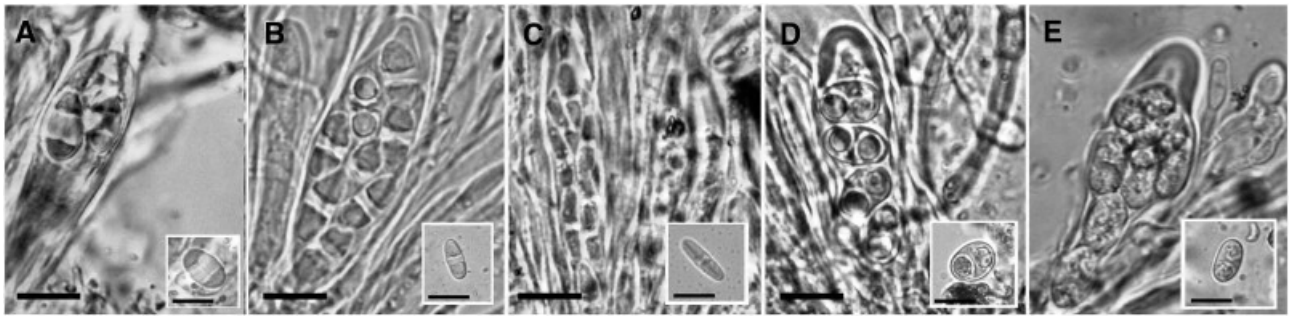

Fig. 1.

Polar-diblastic spores of lichen species belonging to the Teloschistaceae and occurring at the Castle of Graines: spore packages within asci and (inset) asci-free spores. *C. crenularia* (A, length × width, septum length: 13.0 × 8.0, 4.0–5.0 µm); *C. crenulatella* (B, 13.5–16.5 × 6.0–7.5, 2.0–2.5 µm); *C. rubroaurantiaca*(C, 13.0–;15.5 × ; 4.0–;5.0, 0.0–;1.5 µm); *C. saxicola* s.l. (D, 11.5 × 6–7, 1.0–1.5 µm); *X. elegans* (E, 9–10 × 5, 1.5–2.0 µm). Bars = 10 µm.

2.2. Meteorological and microclimatic monitoring

Meteorological data (temperature, relative humidity, rainfall, wind speed and wind direction, photosynthetic active radiation) were obtained from June 22nd 2011 to June 21st 2012 using an in situ station CR1000 (Campbell), equipped with a sonic anemometer (8100 Young), a termo-hygrometric detector (Hygroclip-S3 Rotronic), a PAR detector (SQ1000 Apogee) and a pluviometer, and connected to a GSM/GPRS modem (TecnoEL, Rome) which registered and transmitted 6 hourly readings (map in Supplementary materials 1; monthly climate data in Supplementary materials 3).

2.3. Aerobiological monitoring

Air samples were continuously collected (10 L min^{-1}) one week per month during the period between June 2011 and May 2012 using a 7-day recording Hirst-type volumetric sampler VPPS 2010 (Lanzoni, Bologna), located at 1.5 m above ground level in the middle of the Castle court. Each month, the adhesive-tape on which airborne particles had been impacted was sectioned into lengths (48 mm) representing the monitored days (day 1, from 12 a.m. to 12 p.m.; days 2–7, 24 h; day 8, from 12 p.m. to 12 a.m.), stained with basic fuchsin in a glycerine-jelly medium and mounted on slides to be examined under microscope. Each slide was divided into four vertical fields, corresponding to 6 h each, and examined at 400 × magnification with a Nikon Eclipse 50i equipped with digital camera, counting spores along five traverses 2 mm apart (UNI 11108:2004 in Travaglini et al., 2009).

The total atmospheric spore content was calculated one day per month (day 3). All fungal spores were classified by appearance (color, shape) and morphological features (septa) into 7 physionomic categories: A1, hyaline circular spores; A2, dematiaceous circular spores; A3, hyaline elongate/ellipsoidal spores; A4, dematiaceous elongate/ellipsoidal spores; B1, bicellular spores; B2, multicellular uniseriate spores; B3, multicellular muriform spores. Conidia of *Cladosporium* and *Alternaria* were separately classified.

The concentrations of polar-diblastic spores were calculated 6 days per month (days 2–7). Each spore was characterized in terms of length, width and septum width, and the identification was attempted with reference to the spore characteristics of the Teloschistaceae species occurring in the site.

2.4. Statistical analyses of field data

The Spearman correlation coefficient was used to check for possible associations between spore counts and climate factors. The matrices of spore counts (4 readings of 6 h for each month in the case of total spore counts; 24 readings of 6 h for each month in the case of the counts of polar-diblastic spores) and meteorological data (36 readings averaged for each interval of 6 h considered for spore counts) were processed using Systat 10.2 (Richmond, California).

2.5. Laboratory experiments on spore dispersal pathways

Four groups of rock blocks colonized by *C. rubroaurantiaca*, having small spore size (spores: 13–15 × 4–5 µm; thallus surfaces: 15 and 20 cm^2 on the first and second block groups, respectively), or *Rhizocarpon geographicum* s.l., a dominant species in the study site having big spores (dark green muriform spores: 22–40 × 10–19 µm; thallus surfaces: 65 and 200 cm^2 on the third and fourth block groups, respectively), were sampled around the castle in April 2012. Each group of blocks was incubated for one week within a perspex box (120 × 70 × 60 cm^3) including: the VPPS 2010 sampler (Lanzoni), a fan (FT30-G1, Vigliettam S.P.A., Cuneo, Italy) moving the air at about 4 m sec^{-1} on the surface of the rocks and 10 Petri dishes (d = 5.5 cm), containing an agar medium and surrounding the rocks. All the blocks were immersed in deionized water for 15 min before the incubation, according to preliminary tests which showed this step necessary for observing the spore dispersal. The airborne particles collected with the VPPS were analyzed following the same protocol used for the field monitoring, focusing on the occurrence of *C. rubroaurantiaca* and *R. geographicum* spores. All the slides, corresponding to day 1 to day 8, were analyzed for each weekly incubation. Moreover, the desiccated agar surfaces within the Petri plates were fully examined at 400 × magnification with the Nikon Eclipse 50i microscope. The temperature and relative humidity within the box were monitored using a HOBO®Data Logger U14-001 (Cape Cod, Massachusetts).

3. Results

3.1. Fungal airborne particulate

A total spore concentration of 1372 spores m^{-3} (avg.) was detected through the year, the lowest values characterizing winter months (avg. 214 spores m^{-3}) (Table 1).*Cladosporium* conidia (Clad.) were 53.5% of the total counted spores. Simple hyaline spores with ellipsoidal/ovoid/elongate (A3) or circular (A1) shape were the other dominant components of the mycoaerosol. Polar-diblastic spores of the Teloschistaceae were 0.04% of the overall counts, their average concentration being 0.5 spores m^{-3} (seeSection 3.2). No other ascospores attributable with certainty to lichen-forming fungi, as the dark green muriform spores of *Rhizocarpon*, or symbiotic propagules were observed in the sampled air spora.

Table 1
Fungal spores in the atmosphere of the Castle of Graines between June 2011 and May 2012. A, spore concentration (spores m^{-3}): annual average (avg.) and monthly data; B, contribution of each spore category to the monthly concentrations (%): annual average (avg.) and monthly percentages; C, annual contribution of each spore category to the overall spore concentration (%). Hyal., hyaline; Dem., dematiaceous.

		Unicellular spores				Multicellular spores						Total
		Circular		Elongate		Bicellular	Uniseriate	Muriform	*Cladosporium*	*Alternaria*	Teloschistaceae	
		Hyal. (A1)	Dem. (A2)	Hyal. (A3)	Dem. (A4)	(B1)	(B2)	(B3)	(Clad.)	(Alt.)	(Tel.)	
A	Avg.	119.4	46.1	262.5	96.1	43.4	71.7	15.8	715.2	1.6	0.5	1372.3
	Jun	166.7	108.7	478.8	307.3	36.5	67.0	13.2	2166.0	1.4	0.0	3345.5
	Jul	56.9	78.8	351.0	175.0	36.8	189.6	9.7	736.1	0.0	3.1	1637.2
	Aug	87.5	31.9	152.1	54.2	73.6	97.9	16.3	208.0	5.9	0.0	727.4
	Sep	350.0	157.6	1281.9	206.9	24.3	122.2	110.1	3098.3	9.4	0.0	5360.8
	Oct	108.0	15.3	135.1	22.2	32.3	127.8	8.3	88.9	0.3	3.1	541.3
	Nov	154.2	13.5	255.2	11.5	20.8	4.9	3.5	386.8	0.3	0.0	850.7
	Dec	26.4	11.8	34.7	4.5	9.4	12.2	0.7	156.2	0.0	0.0	255.9
	Jan	72.2	7.6	160.8	2.4	8.0	7.3	1.7	35.1	0.3	0.0	295.5
	Feb	22.9	4.9	18.1	2.4	2.8	2.8	0.0	37.2	0.0	0.0	91.0
	Mar	255.6	13.9	84.7	39.9	11.5	12.2	3.5	93.1	1.4	0.0	515.6
	Apr	36.1	19.8	80.2	36.1	18.4	27.8	1.0	66.0	0.0	0.0	285.4
	May	96.9	88.9	117.4	291.0	246.2	188.9	21.2	1511.1	0.3	0.0	2561.8
B	Avg.	15.9	3.8	22.7	6.1	4.2	6.9	0.8	39.3	0.1	0.1	
	Jun	5.0	3.2	14.3	9.2	1.1	2.0	0.4	64.7	0.0	0.0	
	Jul	3.5	4.8	21.4	10.7	2.2	11.6	0.6	45.0	0.0	0.2	
	Aug	12.0	4.4	20.9	7.4	10.1	13.5	2.2	28.6	0.8	0.0	
	Sep	6.5	2.9	23.9	3.9	0.5	2.3	2.1	57.8	0.2	0.0	
	Oct	19.9	2.8	25.0	4.1	6.0	23.6	1.5	16.4	0.1	0.6	
	Nov	18.1	1.6	30.0	1.3	2.4	0.6	0.4	45.5	0.0	0.0	
	Dec	10.3	4.6	13.6	1.8	3.7	4.7	0.3	61.1	0.0	0.0	
	Jan	24.4	2.6	54.4	0.8	2.7	2.5	0.6	11.9	0.1	0.0	
	Feb	25.2	5.3	19.8	2.7	3.1	3.1	0.0	40.8	0.0	0.0	
	Mar	49.6	2.7	16.4	7.7	2.2	2.4	0.7	18.0	0.3	0.0	
	Apr	12.7	6.9	28.1	12.7	6.4	9.7	0.4	23.1	0.0	0.0	
	May	3.8	3.5	4.6	11.4	9.6	7.4	0.8	59.0	0.0	0.0	
C	Total	8.7	3.4	19.1	7.0	3.2	5.2	1.1	52.1	0.1	0.04	

Most spores were sampled during the afternoon (30% between 12 a.m. and 6 p.m.) and early night hours (32% between 6 and 12 p.m.), while spore counts were lower in the late night hours (21% between 12 p.m. and 6 a.m.) and during the morning (16% between 6 and 12 a.m.). However, the contribution of the different spore categories to the total spore concentrations did not show a significant variability in the different hour intervals considered for the analyses, both examining the total annual counts (Fig. 2) and the single monthly analyses (data not shown).

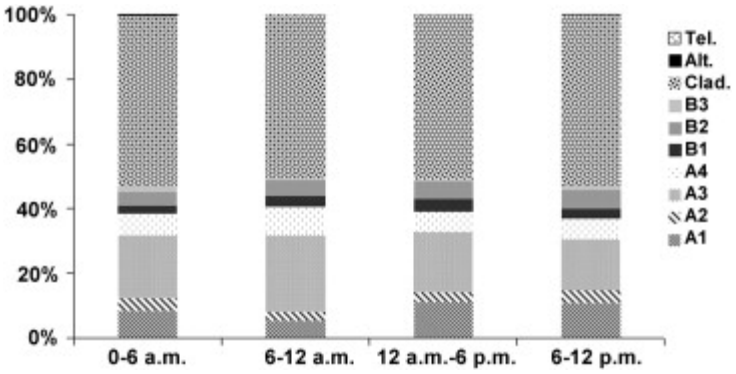

Fig. 2.

Contribution (%) of each spore category to the total spore concentrations detected with reference to the four daily periods of monitoring (0–6 a.m., 6–12 a.m., 12 a.m.–6 p.m., 6–12 p.m.). No significant differences for each spore category between the different daily periods were found according to Tukey's test.

Spearman's rank correlation test analysis showed maximum correlation of *Cladosporium*and dematiaceous spores (A2, A4) with temperature (T) and maximum correlation of multicellular spores (B1–B3) with relative humidity (RH) and rainfall during the sampling period (RG) and the previous 24 h (RD) (Table 2). Hyaline spores (A1–A3) and*Alternaria* (Alt.) also showed a positive correlation with T, as also these spore categories displayed higher concentration in summer with respect to winter, but their relationship with T was lower than that showed by *Cladosporium* and dematiaceous spores. Spores of Teloschistaceae showed the

highest correlation with RD. A redundancy analysis (RDA) of the matrices of total spore counts and meteorological data confirmed the same correlation patterns exhibited by the Spearman's analysis (Supplementary materials 4).

Table 2.

Spearman's rank correlation coefficients (A) between meteorological parameters (T, air temperature; RH, relative humidity; RG, rainfall during the sampling period; RD, rainfall in the previous 24 h; V, wind speed; W, wind direction; PAR, photosynthetic active radiation) and spore categories (abbreviations in Table 1), and (B) between spore categories.

Table 2
Spearman's rank correlation coefficients (A) between meteorological parameters (T, air temperature; RH, relative humidity; RG, rainfall during the sampling period; RD, rainfall in the previous 24 h; V, wind speed; W, wind direction; PAR, photosynthetic active radiation) and spore categories (abbreviations in Table 1), and (B) between spore categories.

A		Meteorological parameters						
		T	RH	PAR	RG	V	W	RD
Spore categories	A1	0.345	–	–	–	–	–	–
	A2	**0.541**	0.441	–	–	–	–	–
	A3	0.343	0.349	–	–	–	–	–
	A4	**0.594**	**0.498**	0.254	–	–	–	–
	B1	0.324	**0.638**	–	0.325	–	–	**0.566**
	B2	0.328	**0.784**	–	0.383	–	–	**0.552**
	B3	**0.498**	**0.593**	–	0.429	–	–	0.268
	Clad.	0.556	**0.244**	–	–	–	–	–
	Alt.	0.400	–	–	–	–	–	–
	Tel.		**0.288**	–	–	−0.297		**0.333**

B		Spore categories									
		A1	A2	A3	A4	B1	B2	B3	Clad.	Alt.	Tel.
Spore categories	A1	**1.000**									
	A2	**0.590**	**1.000**								
	A3	**0.733**	**0.723**	**1.000**							
	A4	**0.536**	**0.905**	**0.632**	**1.000**						
	B1	0.423	**0.647**	0.431	**0.698**	**1.000**					
	B2	0.406	**0.675**	0.508	**0.702**	**0.785**	**1.000**				
	B3	**0.641**	**0.784**	**0.648**	**0.767**	**0.731**	**0.802**	**1.000**			
	Clad.	**0.638**	**0.890**	**0.694**	**0.847**	**0.493**	**0.538**	**0.715**	**1.000**		
	Alt.	**0.534**	0.434	0.404	0.400	0.348	0.331	**0.587**	**0.491**	**1.000**	
	Tel	–	–	–	–	–	0.292	–	–	–	**1.000**

Bold: P < 0.001, underlined: P < 0.01, normal: P < 0.05, – : non-significant.

3.2. Airborne polar-diblastic spores

Polar-diblastic spores were detected in the mycoaerosol sampled in the months of July, August, October and February, a total of 35 spores being counted during 6 days out of the 6-days per month observations (8.3% of monitored days; Table 3). The maximum daily average concentration was observed in two days of July and October (3.1 spores m^{-3}). Most of the spores were sampled during the afternoon (51% between 12 a.m. and 6 p.m.).

Table 3.

Polar-diblastic spores in the atmosphere of the Castle of Graines between June 2011 and May 2012. A, distribution of the spores in the different sampling days and hours; B, assignment of the spores to the different Teloschistaceae species; C, total counts. No spores were observed in the months which are not displayed in the table. (h) = distribution of the counted spores between 0 and 6 a.m./6 and 12 a.m./12 a.m. and 6 p.m./6 and 12 p.m.

		Jul	(h)	Aug	(h)	Oct	(h)	Feb	(h)	Total
A	Day 1	0	–	3	(0/0/0/3)	0	–	0	–	
	Day 2	9	(0/0/9/0)	0	–	9	(0/0/9/0)	0	–	
	Day 3	0	–	0	–	0	–	0	–	
	Day 4	0	–	1	(0/0/0/1)	0	–	0	–	
	Day 5	0	–	0	–	0	–	0	–	
	Day 6	0	–	5	(1/4/0/0)	0	–	8	(8/0/0/0)	
B	*C. rubroaurantiaca*	3		4		8		0		15 (43%)
	C. crenularia	0		0		1		2		3 (9%)
	C. crenulatella	3		4		0		0		7 (20%)
	C. saxicola/*X. elegans*	3		1		0		6		10 (28%)
C	Total	9		9		9		8		35 (100%)

The morphological features of the air-sampled spores, compared with those observed directly in the ascocarps (see Fig. 1), allowed the assignment of each spore to the different species of Teloschistaceae occurring in the investigated site (Fig. 3A–D;Table 3; spore details in Supplementary materials 5).

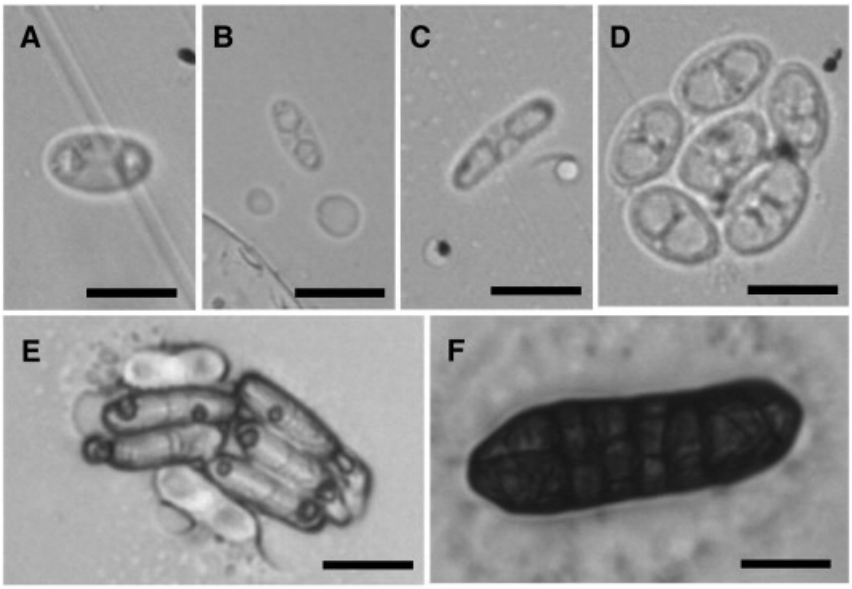

Fig. 3.
Airborne lichen spores. Polar diblastic spores of *C. crenularia* (A), *C. crenulatella* (B), *C. rubroaurantiaca* (C) and *C. saxicola* or *X. elegans* (D) detected in the atmosphere of the Castle of Graines. Polar diblastic spores of *C. rubroaurantiaca* (E) and muriform spore of *R. geographicum* (F) recovered on agar surfaces exposed in the laboratory dispersal tests. Bars = 10 µm.

Thirty-two out of 35 detected polar-diblastic spores were observed less than 8 h after a rainfall (details in Supplementary materials 5). Accordingly, Spearman's rank correlation test analysis (Table 4) showed the

highest correlation of the readings with rainfall of the previous 24 h ($P < 0.001$). Only two rainfall events throughout the monitored period (7 mm in April and 5 mm in May) were not related with the detection of polar-diblastic spores in the air sampled during the following 24 h.

Table 4.
Spearman's rank correlation coefficients between reports of polar-diblastic spores (Tel.) and meteorological parameters (abbreviations in Table 2).

		Tel
Meteorological parameters	T	–
	RH	**0.195**
	PAR	–
	RG	0.131
	V	– 0.143
	W	–
	RD	**0.254**

Bold: $P < 0.001$, underlined: $P < 0.01$, normal: $P < 0.05$, – : non-significant.

The dispersal of polar-diblastic spores was also detected in the laboratory experiments: spores of *C. rubroaurantiaca* were abundantly recovered on the agar surfaces around the incubated rocks (avg. ± SD 1.11 ± 0.05 spores cm^{-2} of surveyed agar). The spores mostly occurred in packages of eight and in several cases displayed hyphal germination (Fig. 3E). Some isolated *Caloplaca* spores were also collected by the Hirst-type volumetric sampler: nine and five spores were detected during the first three days of the two incubation cycles, respectively (avg. ± SD 0.8 ± 0.7 spores m^{-3} during the first three days). It is worth noting, that polar-diblastic spores were not collected neither with the agar plates nor with the volumetric sampler when *Caloplaca*-colonized rocks were incubated without performing the preliminary immersion in water. Isolated *Rhizocarpon*spores (Fig. 3F) were detected on the agar surfaces exposed around the colonized rocks, but their concentration (avg. ± SD 0.027 ± 0.016 spores cm^{-2} of surveyed agar) was strongly lower than that of *Caloplaca* spores and no germination was observed. No muriform, dark-green spores were collected by the volumetric sampler.

4. Discussion

This study first quantifies the concentrations of Teloschistaceae spores in the atmosphere of an alpine environment, highlighting their low percentage occurrence with respect to the whole mycoaerosol (0.04% of total spore counts). Results confirmed our hypothesis that the dispersal of polar-diblastic spores depends on climate factors, their detection being strictly related to rainfall events. Laboratory tests, in agreement with the field monitoring, highlighted different dispersal patterns for small (*C. rubroaurantiaca*) and big (*R. geographicum*) lichen meiospores, but generally suggested a preferential role for short-distance species propagation rather than long-range dispersal.

4.1. Mycoaerosol in alpine environments

Measurements of fungal spore concentrations are on the order of 10^2–10^3 m^{-3}, falling within the lower/intermediate range of the available data on the continental boundary layer air (Fröhlich-Nowoisky et al., 2009 and Sesartic and Dallafior, 2011). Similar spore concentrations, ranging between low values around 400 spores m^{-3} in January and February and high summer concentrations around 3500 spores m^3, were reported byEbner et al. (1989) for urban sites at 600–800 m a.s.l. in the Austrian Alps, while high altitude sites at approx. 1900 m a.s.l. displayed lower concentrations. *Cladosporium*, the dominant element

in the air spora in almost every part of the world (Ingold, 1965), and*Alternaria* also showed annual concentration trends similar to those reported for the alpine sites in Austria, with winter minima below 100 spores m^{-3} and summer maxima higher than 2000 spores m^{-3} for the first taxon and constant low values for the second one (Ebner et al., 1989). To the best of our knowledge, no more comparisons are available for alpine environments, which were poorly examined till now in terms of mycoaerosol because of their poor relevance to allergenic and phytopathogenic issues. Nevertheless, the strong similarity of the detected fungal spore concentrations in the study site with previous data by Ebner et al. (1989) highlights the representativity of the performed monitoring of alpine mycoaerosol and its suitability for disclosing the occurrence of lichen meiospores.

4.2. Lichen ascospores in the mycoaerosol

Polar-diblastic spores displayed a minor and occasional occurrence in the monitored air spora. This result likely reflected the very low contribution of *Caloplaca* and *Xanthoria*species (< 1%) to the total lichen cover in the examined site. Moreover, species of Teloschistaceae represent only 7% of the lichen flora in the study area (Nimis and Martellos, 2008). Accordingly, although different species may display different dispersal pathways (see below), the total lichen component may represent a more significant part of the whole mycoaerosol, which indeed also includes a high percentage of hyaline unicellular spores potentially attributable to other common lichen-forming fungi in the site as *Aspicilia* s.l., *Lecanora* s.l., *Lecidea* s.l. and *Xanthoparmelia* s.l. On the other hand, BLAST similarity analyses of DNA sequences from mycoaerosol in central Europe displayed only two OTUs, out of 362, attributable to lichen-forming fungi (Fröhlich-Nowoisky et al., 2009). Anyway, different environments, displaying different lichen abundance, may be likely related to different abundance of lichen ascospores: both field and laboratory measures highlighted a strong limitation in the spatial dispersal of lichen spores.

On the basis of their morphological features, all the detected polar-diblastic spores were confidently attributable to *X. elegans* or to the four *Caloplaca* species occurring in the site. Although a long-distance wind dispersal was long supposed for lichen spores, opposite to local dispersal of vegetative propagules (Tapper, 1976), our field results did not detect spores of Teloschistaceae species absent from the site. Moreover, in the laboratory experiment, the spores of *C. rubroaurantiaca* sampled in the air volume were isolated and much lower in number than those detected at a centimetric distance from the thalli on the agar plates. On the latter, the spores mostly occurred in packages and a rapid germination was observed, likely minimizing the risk of small spores to be damaged by prolonged desiccation (Chrismas, 1980, Ostrofsky and Denison, 1980 and Pentecost, 1981). These findings suggested that sexual propagules of Teloschistaceae play a major role in local dispersion and community expansion around founder individuals, according to the “signs of local aggregation” generally reported by Hedenås et al. (2003) for spore-dispersed species. Alternatively, although wind was indicated as long-distance dispersal vehicle for lichens, explaining floristic affinities among distant landmasses (Muñoz et al., 2004), long range dispersal to establish new communities appears to be a less common event.

As particles with higher diameters always show higher deposition velocities and lower life times in the air than smaller ones (De Nuntiis et al., 2003), a limited air dispersal even more characterizes the big muriform spores of *R. geographicum*. These were only detected in the laboratory tests at a short distance from the colonized rocks and never observed in the volumetric air sample both in the laboratory and in the field. Accordingly, differences in distributional and (re-)colonization patterns of lichen species having different spore sizes were repeatedly reported (see Section 4.4).

The short range dispersal of mycobiont meiospores likely correlates with a higher possibility to encounter compatible photobionts and re-establish the lichenized state. Accordingly, Sanders and Lücking (2002) observed successful relichenization steps on microscope cover slips put on plants with leaves already colonized by foliicolous lichen communities (i.e. the spore production of germination hyphae and their surrounding of free trebouxioid algae encountered upon the substratum). Moreover, in species pairs (see Mattsson and Lumbsch, 1989, with refs. therein), the sexually reproducing taxa display small distribution areas, while the ones which are dispersed exclusively by symbiotic propagules, without the necessity to encounter a compatible partner, invade large areas, e.g. characterizing the (re-)colonization of postglacial areas (Honegger, 1993 and Mattsson and Lumbsch, 1989). Accordingly, more soredia than ascospores were trapped in aerobiological analyses performed in periglacial areas of maritime Antarctic, higher dominance of asexual propagules mostly characterizing sampling sites with less mature lichen communities (Marshall, 1996). However, from temperate to extreme conditions, species with symbiotic propagules are lower in number than those producing meiospores: these latter not only support dispersal, but also generate novel genetic diversity within populations and possibly enable escape from parasites (Seymour et al., 2005). Accordingly, the absence of soredia in the monitored mycoaerosol not only confirmed the limited dispersal of propagules large in size, but also agreed with the fact that only 7 non-dominant species in the sampling site (13% of total flora; Piervittori et al., 1991) produce asexual propagules.

4.3. Climatic influences

Together with temperature, precipitation primarily controls lichen growth rates (Eaton and Ellis, 2012) and the composition of lichen communities (Aragón et al., 2012, Kidron and Temina, 2013, Maphangwa et al., 2012 and Marini et al., 2011). Rainfall, fog and dew are known to play a crucial role in the biological activity of poikilohydric organisms as lichens, affecting metabolic processes as photosynthesis, gas exchange and nitrogen fixation (Nash, 2008). Our results found that precipitation was also a relevant factor for the dispersal processes of lichen meiospores.

A strong relationship of lichen-spore discharge with rainfall events was already observed through discharge-tests in the laboratory and in the field (Ostrofsky and Denison, 1980 and Pyatt, 1973) and we also observed spore discharge in the laboratory tests only upon a preliminary immersion of the colonized rocks in water. The significant correlation of rainfall events with the detection of Teloschistaceae spores in the mycoaerosol during the subsequent 24 h first highlights that the weather rather than the seasons, mainly related with different temperatures, controls spore dispersal from the permanently fertile lichen ascocarps. Different rainy seasonal patterns likely account for different spore discharge patterns reported around the globe (see Bailey, 1976 and Pyatt, 1973). The distribution of the polar-diblastic spore counts during the day also appeared more correlated with the daily distribution of rainfall events than with biological mechanisms, not confirming previous reports on well-defined nocturnal rhythms of spore discharge (Pyatt, 1973). *Caloplaca* and *Xanthoria* spores thus showed a different dispersal pattern with respect to that of dominant *Cladosporium* and *Alternaria* conidia and of dematiaceous simple spores (A2, A4), showing a positive correlation with temperatures and dry conditions, commonly reported throughout the world (e.g. Troutt and Levetin, 2001). Accordingly, different relationships of different fungal species with climate factors were previously reported (Li and Kendrick, 1995 and Trejo et al., 2012).

Higher rates of lichen spore dispersal under rainy rather than dry climates may explain positive correlations of duration and quantity of rainfall or dew availability to species richness, unequivocally related till now with physiological requirements of the symbiotic partners (Kidron and Temina, 2013 and Maphangwa et al.,

2012). Rainy regions not only exhibit longer periods with atmospheric humidity suitable for growth and reproduction (Marini et al., 2011), but also offer favorable conditions for the dispersal step. This confirms the hypothesis by Aragón et al. (2012) that lower specific diversity and higher levels of similarity (lower specific turnover) between epiphytic lichen communities at low latitudes are related to more stressful conditions, but also depend on a potential higher limitation by species dispersal. Accordingly, predicted changes in climate, including precipitation, will affect lichen communities not only because of their effects on the symbiotic carbon balance (Gómez-Bolea et al., 2012 and Maphangwa et al., 2012), but also because of their influence on propagule dispersal rates.

4.4. Applicative remarks and conclusions

Shifts in the composition of lichen communities have been related to different drivers, including pollution, climatic change and human management (Ellis and Coppins, 2010), being of interest for environmental monitoring and diversity conservation. Colonization-extinction and recolonization patterns have been examined following changes in air quality (Nimis et al., 2002) or landscape variations, as glacial retreats (Raab et al., 2012) or forest disturbances (Nascimbene et al., 2012), and simulating climate change (e.g.Maphangwa et al., 2012). Very poor attention, however, has been pointed on the dependence of these processes on the dispersal dynamics of lichen species. Our results on the precipitation influence on Teloschistaceae dispersal further link lichens and environmental features, increasing the significance of lichens as biomonitors of climate change. On the other hand, the low occurrence of polar diblastic spores in mycoaerosol and the highlighted dominance of short-range dispersal patterns suggest that dispersal limitations at small scales may spatially (or, at least, temporally) limit the response of lichens to changes in bioclimatic spaces, as already modeled for the epiphytic species*Vulpicida pinastri* (Binder and Ellis, 2008). Moreover, highlighted differences in the dispersal of small (Teloschistaceae) and big (*Rhizocarpon*) spores support the recurrent hypothesis that the entity of dispersal limitations and consequent distributional patterns depend on the reproductive traits: sexually reproducing species with small spores were shown to more closely track the distribution of their potential substrate than species with large meiospores or asexual propagules (Binder and Ellis, 2008), and to prevail in first successional stages in deglaciated environments (e.g. Favero-Longo et al., 2012), disturbed areas (e.g. Sparrius et al., 2012) and pollution-related lichen deserts (Sparrius, 2012). Lichens with different reproductive traits can be thus confidently considered as indicators of environmental changes having different spatial (and temporal) efficiency, species with small meiospores likely displaying more rapid responses to modified conditions.

In the different context of cultural heritage conservation, lichen colonization is generally considered a significant threat for stonework (St. Clair and Seaward, 2004) and lichen airborne particulate has been supposed as a potential factor of biodeterioration (Favali et al., 2003). Our insights on the lichen dispersal dynamics suggest that, for a long-term success of cleaning procedures (see Nascimbene et al., 2009), physical and/or chemical treatments on the target stone surfaces should be extended to neighboring lichen communities which may support recolonization with their direct, short-range spore discharge (see Pyatt, 1973), while immigration of lichen spores from long distances may appear less probable. On the other hand, if stonework conservation is not threatened, the removal of lichen communities having value as biodiversity components or bioprotective agents (St.Clair and Seaward, 2004) should be discouraged: poor possibilities of re-establishment dynamics can be expected on a short term and the immigration of more aggressive organisms may be favored.

In conclusion, this quantitative investigation on polar-diblastic spores in the mycoaerosol highlights that lichen aerobiology may support the analyses of community shifts as indicators of environmental changes

and improve management and conservation programs of different lichen substrates, including the stone cultural heritage, by disclosing colonization dynamics. Methods to combine quantitative aerobiological approaches with the possibility of distinguishing all lichen meiospores from those of non-lichenized fungi are now required to extend results beyond the model group of Teloschistaceae and obtain an overall evaluation of lichen species occurrence in the air spora.

Acknowledgments

The authors are grateful to the Editor J.P. Bennett and two anonymous reviewers for the valuable suggestions which improved the effectiveness of the study. This work was carried on with the financial support of the Interreg Program Alcotra 2007–2013-Project: "Anciens vestiges en Ruines (AVER) — Des Montagnes des Châteux". The authors are grateful to Dr. G. Sartorio and S. Migliorini (Soprintendenza per i beni e le attività culturali, Regione Autonoma Valle d'Aosta), to D. Blisa (University of Torino) and to the personnel of F.T. Studio S.r.l. (Torino) and Tecno.el. s.r.l. (Roma) for the assistance during the field work.

References

Aragón et al., 2012 G. Aragón, I. Martínez, A. García **Loss of epiphytic diversity along a latitudinal gradient in southern Europe** Sci Total Environ, 426 (2012), pp. 188–195

Armstrong, 1994 R.A. Armstrong **Dispersal of soredia from individual soralia of the lichen *Hypogymnia physodes* (L.) Nyl. in a simple wind tunnel** Environ Exp Bot, 34 (1994), pp. 39–45

Bailey, 1976 R.H. Bailey **Ecological aspects of dispersal and establishment in lichens** D.H. Brown, D.L. Hawksworth, R.H. Bailey (Eds.), Lichenology: progress and problems, Academic Press, London (1976), pp. 215–247

Binder and Ellis, 2008 M.D. Binder, C.J. Ellis **Conservation of the rare British lichen *Vulpicida pinastri*: changing climate, habitat loss and strategies for mitigation** Lichenologist, 40 (2008), pp. 63–79

Büdel and Scheidegger, 2008 B. Büdel, C. Scheidegger **Thallus morphology and anatomy** T.H. Nash III (Ed.), Lichen biology, Cambridge University Press, Cambridge (2008), pp. 40–68

Chrismas, 1980 M. Chrismas **Ascospore discharge and germination in *Xanthoria parietina*** Lichenologist, 12 (1980), pp. 403–405

Clayden, 1997 S.R. Clayden **Seasonal variation in ascospore discharge in *Rhizocarpon lecanorinum*** Lichenologist, 29 (1997), pp. 495–499

De Nuntiis et al., 2003 P. De Nuntiis, O. Maggi, P. Mandrioli, G. Ranalli, C. Sorlini **Monitoring the biological aerosol** P. Madrioli, G. Caneva, C. Sabbioni (Eds.), Cultural heritage and aerobiology, Kluwer Academic Publishers, Dordrecht (2003), pp. 107–144

Després et al., 2012 V.R. Després, J.A. Huffman, S.M. Burrows, C. Hoose, A.S. Safatov, G. Buryak, *et al.***Primary biological aerosol particles in the atmosphere: a review** Tellus Ser B, 64 (2012), p. 15598

Eaton and Ellis, 2012 S. Eaton, C.J. Ellis **Local experimental growth rates respond to macroclimate for the lichen epiphyte *Lobaria polmonaria*** Plant Ecol Divers, 5 (2012), pp. 365–372

Ebner et al., 1989 M.R. Ebner, K. Haselwandter, A. Frank **Seasonal fluctuations of airborne fungal allergens** Mycol Res, 92 (1989), pp. 170–176

Ellis and Coppins, 2010 C.J. Ellis, B.J. Coppins **Partitioning the role of climate, pollution and old-growth woodland in the composition and richness of lichen epiphytes in Scotland** Lichenologist, 42 (2010), pp. 601–614

Favali et al., 2003 M.A. Favali, F. Gallo, O. Maggi, P. Mandrioli, E. Pacini, G. Pasquariello, *et al.* **Analysis of the biological aerosol** P. Mandrioli, G. Caneva, C. Sabbioni (Eds.), Cultural heritage and aerobiology, Kluwer Academic Publishers, Dordrecht (2003), pp. 145–172

Favero-Longo et al., 2012 S.E. Favero-Longo, W.R. Worland, P. Convey, R.I. Lewis Smith, R. Piervittori, M. Guglielmin, *et al.* **Primary succession of lichen and bryophyte communities following glacial recession on Signy Island, South Orkneys Island Maritime Antarctic** Ant Sci, 24 (2012), pp. 323–336

Fröhlich-Nowoisky et al., 2009 J. Fröhlich-Nowoisky, D.A. Pickersgill, V.R. Després, U. Pöschl **High diversity of fungi in air particulate matter** PNAS, 106 (2009), pp. 12814–12819

Gómez-Bolea et al., 2012 A. Gómez-Bolea, E. Llop, X. Ariño, C. Saiz-Jimenez, A. Bonazza, P. Messina,*et al.* **Mapping the impact of climate change on biomass accumulation on stone** J Cult Herit, 13 (2012), pp. 254–258

Hedenås et al., 2003 H. Hedenås, V.O. Bolyukh, B.G. Jonsson **Spatial distribution of epiphytes on *Populus tremula* in relation to dispersal mode** J Veg Sci, 14 (2003), pp. 233–242

Honegger, 1993 R. Honegger **Developmental biology of lichens. Tansley review no. 60** New Phytol, 125 (1993), pp. 659–677

Ingold, 1965 C.T. Ingold **Spore liberation** Clarendon Press, Oxford (1965)

Kidron and Temina, 2013 G.J. Kidron, M. Temina **The effect of dew and fog on the lithic lichens along an altitudinal gradient in the Negev Desert** Geomicrobiol J, 30 (2013), pp. 281–290

Li and Kendrick, 1995 D.W. Li, B. Kendrick **A year-round study on functional relationships of airborne fungi with meteorological factors** Int J Biometeorol, 39 (1995), pp. 74–80

Maphangwa et al., 2012 K.W. Maphangwa, C.F. Musil, L. Raitt, L. Zedda **Experimental climate warming decreases photosynthetic efficiency in lichens in an arid South African ecosystem** Oecologia, 169 (2012), pp. 257–268

Marini et al., 2011 L. Marini, J. Nascimbene, P.L. Nimis **Large-scale patterns of epiphytic lichen species richness: photobiont-dependent response to climate and forest structure** Sci Total Environ, 409 (2011), pp. 4381–4386

Marshall, 1996 W.A. Marshall **Aerial dispersal of lichen soredia in the maritime Antarctic** New Phytol, 134 (1996), pp. 523–530

Mattsson and Lumbsch, 1989 J.-E. Mattsson, H.T. Lumbsch **The use of the species pair concept in lichen taxonomy** Taxon, 38 (1989), pp. 238–241

Muñoz et al., 2004 J. Muñoz, A.M. Felicisimo, F. Cabezas, A.R. Burgaz, I. Martínez **Wind as a long-distance dispersal vehicle in the Southern Hemisphere** Science, 304 (2004), pp. 1144–1147

Nascimbene et al., 2009 J. Nascimbene, O. Salvadori, P.L. Nimis **Monitoring lichen recolonization on a restored calcareous statue** Sci Total Environ, 407 (2009), pp. 2420–2426

Nascimbene et al., 2012 J. Nascimbene, P.L. Nimis, R. Benesperi **Mature non-native black-locust (*Robinia pseudoacacia* L.) forest does not regain the lichen diversity of the natural forest** Sci Total Environ, 421–422 (2012), pp. 197–202

Nash, 2008 T.H. Nash III **Lichen biology** Cambridge University Press, Cambridge (2008)

Nimis and Martellos, 2008 P.L. Nimis, S. Martellos **ITALIC. The Information System on Italian Lichens. Version 4.0** University of Trieste, Dept. of Biology (2008)

Nimis et al., 2002 P.L. Nimis, C. Scheidegger, P.A. Wolseley **Monitoring with lichens — monitoring lichens** Kluwer Academic Publishers, Dordrecht (2002)

Ostrofsky and Denison, 1980 A. Ostrofsky, W.C. Denison **Ascospore discharge and germination in *Xanthoria polycarpa*** Mycologia, 72 (1980), pp. 1171–1179

Pentecost, 1981 A. Pentecost **Some observations on the size and shape of lichen ascospores in relation to ecology and taxonomy** New Phytol, 81 (1981), pp. 667–678

Piervittori et al., 1991 R. Piervittori, A. Laccisaglia, L. Appolonia, L.M. Gallo **Aspetti floristico-vegetazionali e metodologici relativi ai licheni su materiali lapidei in Valle d'Aosta** Rev Valdôtaine Hist Nat, 45 (1991), pp. 53–86

Pyatt, 1973 F.B. Pyatt **Lichen propagules** V. Ahmadjian, M.E. Hale (Eds.), The lichens, Academic Press, New York (1973), pp. 117–145

Raab et al., 2012 T. Raab, J. Krümmerbein, A. Schneider, W. Gerwin, T. Maurer, A. Naeth **Initial ecosystem processes as key factor of landscape development — a review** Phys Geogr, 33 (2012), pp. 305–343

Sanders and Lücking, 2002 W.B. Sanders, R. Lücking **Reproductive strategies, relichenization and thallus development observed in situ in leaf-dwelling lichen communities** New Phytol, 155 (2002), pp. 425–435

Schoch et al., 2009 C.L. Schoch, G.H. Sung, F. López-Giráldez, J.P. Townsend, J. Miadlikowska, V. Hofstetter, *et al.* **The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits** Syst Biol, 58 (2009), pp. 224–229

Sesartic and Dallafior, 2011 A. Sesartic, T.N. Dallafior **Global fungal spore emissions, review and synthesis of literature data** Biogeosciences, 8 (2011), pp. 1181–1192

Seymour et al., 2005 F.A. Seymour, P.D. Crittenden, P.S. Dyer **Sex in the extremes: lichen forming fungi** Mycologist, 19 (2005), pp. 51–58

Sparrius, 2012 L.B. Sparrius **Lichen conservation in densely populated areas: pressures and measures** Lichen from genome to ecosystem in a changing world, The 7th symposium of the International Association of Lichenology, Ramkhamhaeng University, Bangkok (2012)

Sparrius et al., 2012 L.B. Sparrius, J. Sevink, A.M. Kooijman **Effects of nitrogen deposition on soil and vegetation in primary succession stages in inland drift sands** Plant Soil, 353 (2012), pp. 261–272

St. Clair and Seaward, 2004 L.L. St. Clair, M.R.D. Seaward **Biodeterioration of stone surfaces** Kluwer Academic Publishers, Dordrecht (2004) [Dordrecht]

Tapper, 1976 R. Tapper **Dispersal and changes in the local distributions of *Evernia prunastri* and *Ramalina farinacea*** New Phytol, 77 (1976), p. 725.34

Tormo et al., 2001 R. Tormo, D. Recio, I. Silva, A.F. Munõz **A quantitative investigation of airborne algae and lichen soredia obtained from pollen traps in South-west Spain** Eur J Phycol, 36 (2001), pp. 385–390

Travaglini et al., 2009 A. Travaglini, R. Albertini, E. Zieger **Manuale di gestione e qualità della Rete Italiana di Monitoraggio in Aerobiologia R.I.M.A.** Ozzano Emilia, L.E.G.O. s.p.a. Calderini (2009)

Trejo et al., 2012 F.H. Trejo, A.F. Muñoz Rodríguez, R. Tormo Molina, Palacios I. Silva **Airborne ascospores in Mérida (SW Spain) and the effect of rain and other meteorological parameters on their concentration** Aerobiologia, 28 (2012), pp. 13–26

Troutt and Levetin, 2001 C. Troutt, E. Levetin **Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma** Int J Biometeorol, 45 (2001), pp. 64–74

Verger, 1992 J.P. Verger **Vegetation and soils in the Valle d'Aosta (Italy)** A.J.M. Baker, J. Proctor, R.D. Reeves (Eds.), The vegetation of ultramafic (serpentine) soils, Intercept, Andover (1992)